Appendix

[Amended part in page 14 of the specification. Inserted sentences are underlined]

Figure 1 shows construction of an expression vector for an antithrombin (AT) recombinant variant (in case of Ser380His).

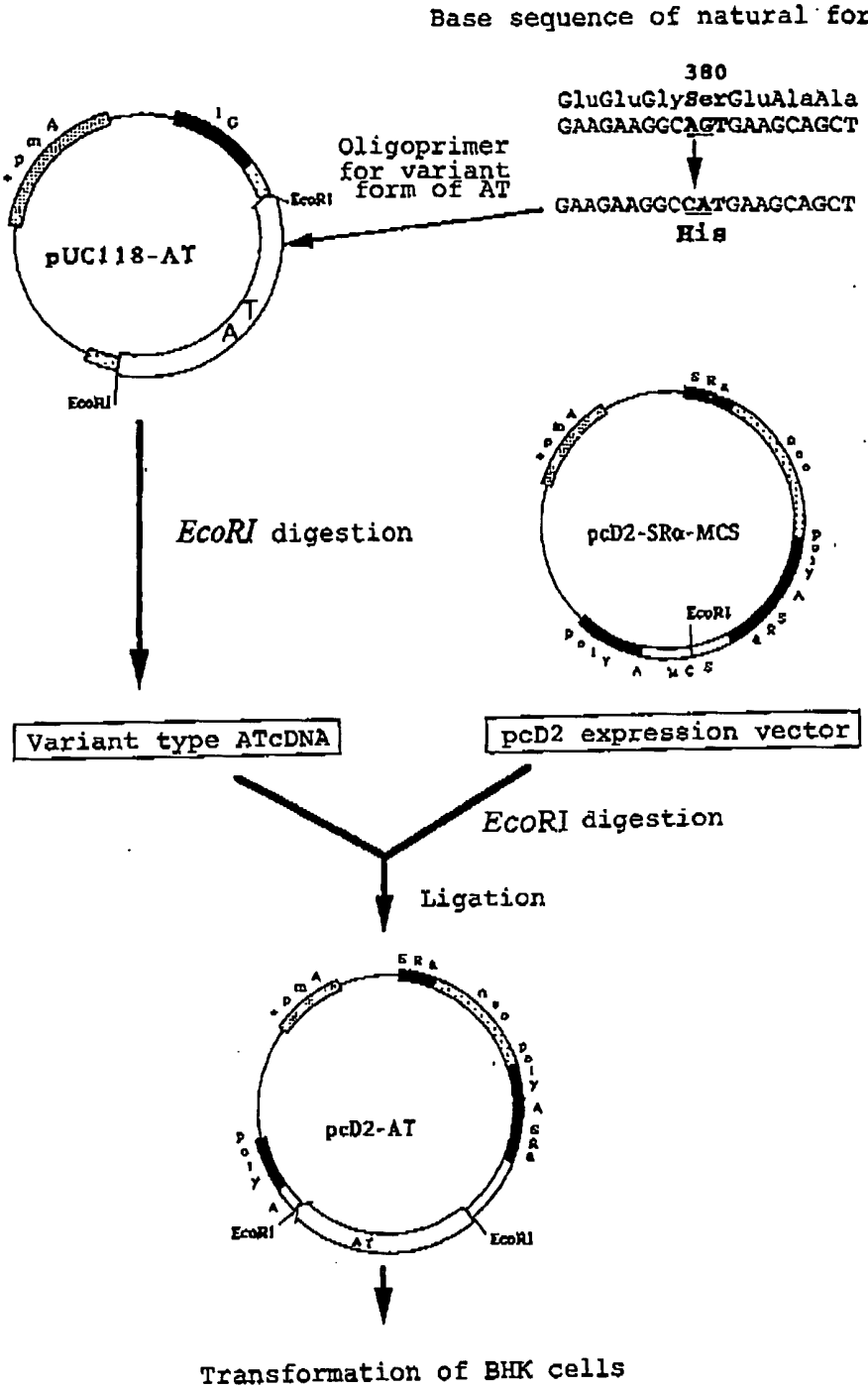
[DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS]

The novel antithrombin variant of the invention was prepared by site-specific mutagenesis as a variant having a three dimensional structure, which is similar to that after binding with heparin. Namely, a cDNA coding the antithrombin variant was prepared by (1) preparation of a single-strand pUC118-AT, (2) introduction of mutation with Sculptor method, (3) confirmation of mutation insertion, and (4) EcoRI digestion. The variant cDNA was inserted into EcoRI-digested pcD2 expression vector. The resultant plasmid was used for transfection of BHK cells. The transfected BHK cells were selectively cultivated to produce the antithrombin variant (Fig.1).

The invention is more specifically illustrated by way of the specific method for the preparation of a variant as set forth hereinafter.

To 2.5 μg (10 μl) of cDNA (single-stranded) for the native form of antithrombin was annealed 30 μl of a variant primer (0.475 OD/ml) for amino acid substitution to synthesize a full-length cDNA with DNA polymerase. Then, the nucleotide sequence was determined to confirm a formation of variant. cDNA (1.4kb) for each antithrombin variant was integrated into EcoRI site of pcD2 vector and then cleaved with EcoRI and PstI to confirm the direction of the inserted sequence. The vector with sequence integrated in right direction was transfected into BHK cells for a large-scale production by a calcium phosphate method (FIG. 1).

FIG. 1


Base sequence of natural form of AT
380
GluGluGlySerGluAlaAla
GAAGAAGGCAGTGAAGCAGCT
Oligoprimer for variant form of AT
GAAGAAGGCCATGAAGCAGCT
His
pUC118-AT
EcoRI
EcoRI
EcoRI digestion
pcD2-SRα-MCS
EcoRI
Variant type ATcDNA
pcD2 expression vector
EcoRI digestion
Ligation
pcD2-AT
EcoRI
EcoRI
Transformation of BHK cells